# Sex ratio and frequency of osteological abnormalities in the Australian hylid frog *Litoria aurea* from two apparently unpolluted localities in Sydney, New South Wales

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**ABSTRACT**

Metamorphling specimens of the endangered frog *Litoria aurea* were sampled from two relatively unpolluted sites in the metropolitan Sydney area for baseline information on sex ratio, vertebral number, vertebral and sacral diapophysis asymmetry, and phalangeal formula. The sex ratio was not significantly different from parity. Vertebral number was a constant eight which is typical for the family Hylidae. There was a vertebral diapophysis asymmetry in one specimen from one population (frequency: 2.4%). The only phalangeal abnormality detected was the late ossification of the terminal phalange in the digits 2–4 of the right manus of one specimen in the other population (frequency: 2.8%).

## INTRODUCTION

Amphibians are considered to be useful indicator species for monitoring the impact of pollution due to their aquatic larval stages and their generally permeable skins (Duellman and Trueb 1986; Blaustein and Wake 1990; Tyler 1991). In order to assess whether an environmental factor may have a deleterious effect on local amphibian populations, the natural baseline variation in key biological features of populations from relatively unpolluted environments has to be established. The purpose of this note is to provide such information on the sex ratio and several osteological features in the manus and pes, and in the vertebral column for two Sydney populations (Fig. 1) of the Green and Golden Bell Frog *Litoria aurea* (family Hylidae).

*Litoria aurea* ranges along the east coast of Australia from northeastern New South Wales to eastern Victoria where it occurs in fairly open habitats with relatively still bodies of water for breeding (Moore 1961; Courtice and Grigg 1975; Barker and Grigg 1977; Hero *et al.* 1991; Cogger 1992; Tyler 1992; Robinson 1991, 1993). The frog was relatively common until about the mid-1960s (Cogger 1960; J. Cann, pers. comm.; T. Boylan, pers. comm.), but it is now listed as a threatened species on Schedule 12 of the New South Wales *National Parks and Wildlife Act* 1974 as amended by the *Endangered Fauna (Interim Protection) Act* 1991, as published February 1992 (Lunney and Ayers 1993; see also White 1993a, b). This ranking places it among the most endangered species in New South Wales. As a result it is an important species in much of the environmental survey work that is required to be carried out in New South Wales prior to issuing a development permit.

## METHODS

The two populations of *Litoria aurea* were from the Sydney metropolitan area (Fig. 1). One population was from a site at the corner of Dalmeny Avenue and Kimberley Grove (Cogger *et al.* 1993; Fig. 2) in Rosebery, and the other was from a site adjacent to the now levelled abattoir at Homebush Bay, colloquially known as "Lake Domis" (Greer 1994; Fig. 3). The Rosebery site is a permanent pond on sand, and the Homebush Bay site is a transient pond on a mixture of building debris and earth. The Rosebery site is just 5.2 km from the Sydney GPO and is the most urban of the 16 known Sydney locations for the frog (White 1993a).

Both populations were represented by very recently metamorphosed specimens: 35 from Rosebery collected on 16 January 1994 (Australian Museum R 143509–143543) and 42 from Homebush Bay collected on 19 December 1993 (AM R 143078–143119). All specimens were collected under New South Wales National Parks and Wildlife Scientific Authority No. A 1344 in conjunction with a project to assess the genetic variability within the various Sydney populations of the species.

The variables examined in the two populations were sex ratio and the following osteological features: number of phalanges in both the manus and pes; number of presacral vertebrae; and general shape of the vertebral


*Fig. 1.* Map of greater Sydney area showing the location of the two study sites: Homebush Bay in the west and Rosebery in the south-east (black dots). Map courtesy of NRMA – 1995.

diapophyses. Sex was determined by gross examination of the gonad. This method was confirmed by histological examination of the gonad in eight individuals from one of the two populations (AM R 143511, 143515, 143522, 143527, 143532, 143535, 143537, 143540). For histology, the gonads were fixed in 10% buffered formalin for 2–3 days and stored in 70% ethanol. The tissues were dehydrated in graded ethanols, embedded in paraffin and serially sectioned at 7 mm. These sections were stained with haematoxylin and eosin. The osteological features were studied through radiographs.

Chi-square was used to compare sex ratios and Student's "t" tests were used to compare means in snout-vent lengths. A probability level of 0.05 was accepted as significant.

## RESULTS

Gross examination of the gonads of the recently metamorphosed frogs revealed two different shapes to the organ: a compact ovoid; and a more elongate and slightly convoluted ribbon. On the basis of the size and shape of the mature gonad, the former shape was interpreted as a testis and the latter as an ovary. This interpretation was confirmed by histological examination of eight gonads. The Rosebery population had 18 males and 16 females and one indeterminate ($X^2 = 0.12$, NS) and the Homebush population had 20 males

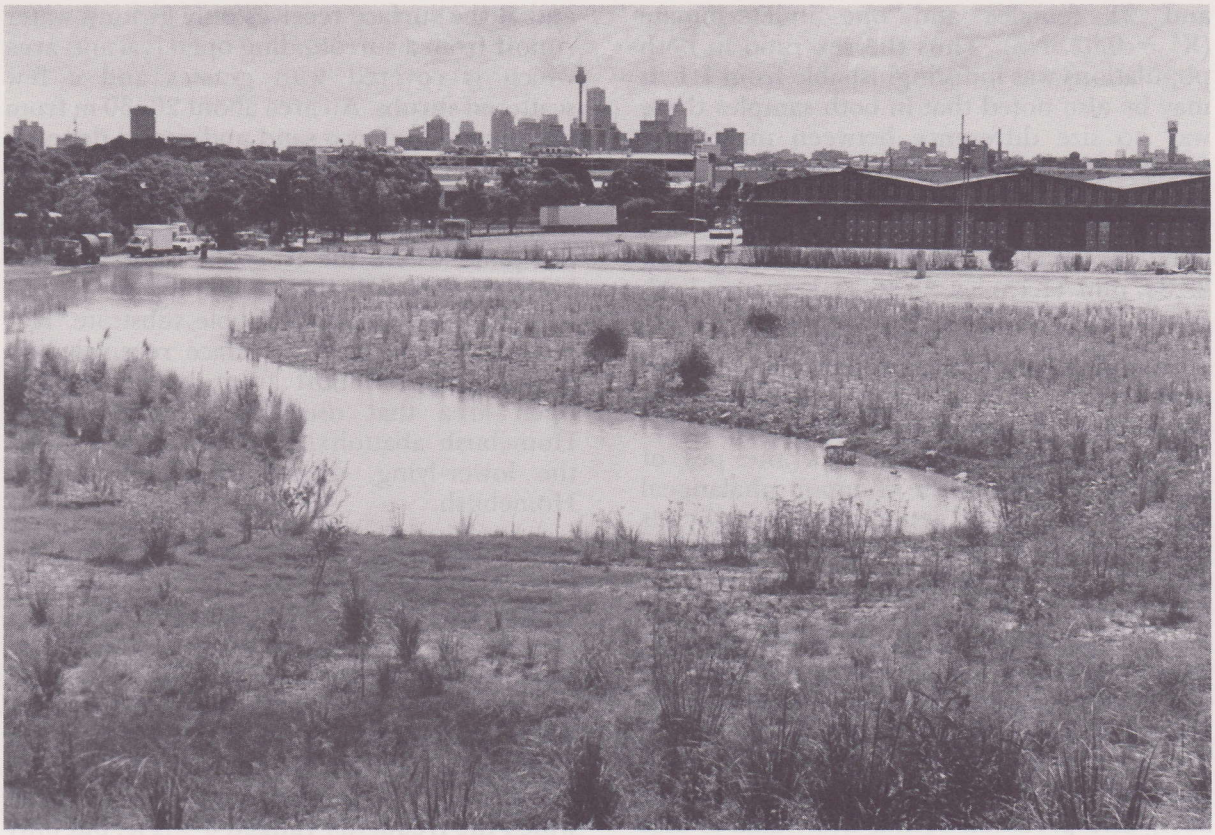

*Fig. 2.* The breeding pond for *Litoria aurea* at the Rosebery site (corner of Dalmeny Avenue and Kimberley Grove). Photo: the author. Date: January, 1994.

*Fig. 3.* The breeding pond for *Litoria aurea* at "Lake Domis" on the former site of the abattoirs at Homebush Bay. Photo: the author, Date: January, 1994.

and 21 females and one indeterminate ($X^2$ = 0.02, NS). Thus the sex ratio in both populations was indistinguishable from 1:1. It may be also noted that in both samples there was no size difference between males and females in snout-vent length (mean and standard deviation for males and females at Rosebery: 26.82 mm (1.11) vs 26.76 mm (1.60), t = 0.13, P = 0.90) and at Homebush: 28.84 mm (1.15) vs 28.36 (1.13), t = 1.36, P = 0.18).

Examination of the radiographs revealed that all but one of the specimens had a phalangeal formula for the manus of 2.2.3.3 and a phalangeal formula for the pes of 2.2.3.4.3. These are the common phalangeal formulas for frogs in general, including hylids (Duellman and Trueb 1986). The single exception was a male from the Rosebery population (AM R 143515) in which the terminal phalanges of digits 2–4 of the right manus were not evident, although those of digit 1 of the right manus (albeit only faintly) and all those of the left manus were evident. The digits of the right manus of this specimen were normal in gross appearance. The most likely interpretation of this individual is that it showed late ossification of the terminal phalanges of the right manus. Thus the level of abnormality in the phalanges was nil in the Homebush population and 2.8% in the Rosebery population.

The number of presacral vertebrae in all specimens from both populations is eight. This is the usual number of presacral vertebrae in hylid frogs (Duellman and Trueb 1986).

The shape of the vertebral diapophyses showed only one peculiarity in one individual. A male from the Homebush Bay population (AM R 143082) had the left diapophysis of the most posterior presacral vertebra expanded to resemble the diapophysis of the following sacral vertebra (the right side was obscured by opaque material in the lower gut). Thus the level of abnormality in the shape of the vertebral diapophyses was nil in the Rosebery population and 2.4% in the Homebush population.

## DISCUSSION

Despite the metropolitan location of the two sample sites (Fig. 1) there was no obvious pollution affecting either location. Specifically, there was no visual or olfactory indication of petroleum pollution, and there was no algal bloom which would indicate nutrient pollution. The breeding pool at the Rosebery site (Fig. 2) is on a sand substrate. It is a permanent pool and at the surface receives only ground water runoff from a surrounding open (vacant) area which is covered with grasses and a few scattered shrubs. An area about 20–30 m from the pool served as a sand and gravel depot at the time of the study. Although this depot had extensive truck and heavy machinery traffic, there was no obvious petroleum pollution into the pond where the frogs are resident. The breeding pool at the Homebush Bay site (Fig. 3) is on an earth/building rubble substrate. It is temporary and at the surface receives only ground water runoff from a surrounding open area that used to be part of the Homebush abattoirs. This site is well above the lower-lying, contaminated fill sites at Homebush.

The frequency of abnormalities in the two populations of *Litoria aurea* (≤ 2.8%) is similar to the frequency of abnormalities in other frog species in other relatively low pollution-level environments around the world. A recent world review of the frequency of limb abnormalities in frog populations "not exposed to pollution of any kind" (Tyler 1989) revealed nine cases involving 16 species in which the frequency ranged 0–3.1%, but only one of these was higher than 3%. In Australia, two recent analyses of limb abnormalities in the myobatrachid frog *Neobatrachus centralis* from 12 sites near Roxby Downs revealed levels of grossly detectable abnormalities (i.e., externally visible under a dissecting microscope) ranging from 0 to 9.0%, the highest level (9.0%) coming, surprisingly, from a locality used as the reference site for other sites thought to be possibly polluted (frequency of 0 to 3.5%; Read and Tyler 1990, 1994). This anomalously high frequency was not discussed further. In the Sydney region, a survey of a population of the hylid frog *Litoria dentata* at a locality near Richmond, New South Wales, revealed a frequency of limb abnormalities not due to injury of 1.3% (Ferraro and Burgin 1993).

In addition to occurring in only relatively low frequency, the abnormalities described here for the two *Litoria aurea* populations are mild. They would not have been detected grossly, the criterion used for the "abnormal" assessment in the Roxby Downs analysis, and they are also unlikely to have been debilitating to the frogs.

In conclusion, it appears that in these two, apparently only slightly "polluted", or unpolluted, metropolitan localities, recently metamorphosed *L. aurea* show a balanced sex ratio and a low frequency (≤2.8%) of mild

limb and vertebral abnormalities. It is intended that these data should now provide a useful baseline against which populations of *L. aurea* from other environments can be examined.

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